QT PRODACT: In Vivo QT Assay With a Conscious Monkey for Assessment of the Potential for Drug-Induced QT Interval Prolongation

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Abstract. In safety pharmacology studies, the effects on the QT interval of electrocardiograms are routinely assessed using a telemetry system in cynomolgus monkeys. However, there is a lack of integrated databases concerning in vivo QT assays in conscious monkeys. As part of QT Interval Prolongation: Project for Database Construction (QT PRODACT), the present study examined 10 positive compounds with the potential to prolong the QT interval and 6 negative compounds considered to have no such effect on humans. The experiments were conducted at 7 facilities in accordance with a standard protocol established by QT PRODACT. The vehicle or 3 doses of each test compound were administered orally to male cynomolgus monkeys (n = 3 – 4), and telemetry signals were recorded for 24 h. None of the negative compounds prolonged the corrected QT using Bazett’s formula (QTcB) interval. On the other hand, almost all of the positive compounds prolonged the QTcB interval, but haloperidol, terfenadine, and thioridazine did not. The failure to detect the QTcB interval prolongation appeared to be attributable for the differences in metabolism between species and/or disagreement with Bazett’s formula for tachycardia. In the cynomolgus monkeys, astemizole induced Torsade de Pointes and cisapride caused tachyarrhythmia at lower plasma concentrations than those observed in humans and dogs. These results suggest that in vivo QT assays in conscious monkeys represent a useful model for assessing the risks of drug-induced QT interval prolongation.

Keywords: safety pharmacology, cynomolgus monkey, telemetry assay, QTc interval, QT PRODACT

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Introduction

Safety pharmacology studies are defined as investigations into potentially undesirable pharmacodynamic effects of a test substance on the physiological functions after exposure to the therapeutic dose and above (1).

The S7A safety pharmacology guideline was agreed upon in November 2001 as the all-inclusive principles for safety pharmacological studies. The S7A guideline stipulates the core battery tests for investigating the effects of a test substance on vital functions, including the central nervous system, cardiovascular system, and respiratory system. This guideline recommends the use of unrestrained animals that may be chronically instrumented with a telemetry transmitter. The core battery tests for the cardiovascular system also require the evaluation of repolarization and conductance abnormalities of the ventricles in addition to blood pressure and heart rate (HR).

Since there is no sufficient consensus on the evaluation of repolarization-associated ventricular tachyarrhythmia (e.g., Torsade de Pointes (TdP)), a rational consensus approach for the preclinical evaluation of the TdP hazard and risk assessment is required. The S7B safety pharmacology guideline described a consensus to present some currently available methods, discuss their advantages and disadvantages, and recommend testing strategies (2).

In order to contribute to the development of the S7B guideline, QT PRODACT was organized by the Japan Pharmaceutical Manufacturers Association (JPMA), and in vitro and in vivo experiments were conducted to assess drug-induced QT interval prolongation according to routine protocols for safety pharmacology studies. QT PRODACT was originally organized by the former safety pharmacology subgroups of the JPMA, and some members from the Japan Association of Contract Laboratories for Safety Evaluation (JACL) also participated.

The S7B guideline describes a non-clinical testing strategy for assessing the potential of a test substance to delay ventricular repolarization. Prolongation of the cardiac action potential duration (APD), which is reflected in electrocardiograms (ECGs) as QT prolongation, is associated with potentially lethal polymorphic ventricular tachyarrhythmia (e.g., TdP). During the past two decades, it was found that most non-antiarrhythmic drugs that prolong the QT interval inhibit the delayed rectifying potassium current, $I_{Kr}$, which is encoded by the human ether-a-go-go related gene (hERG). The S7B guideline introduces a hERG current assay as one of the reasonable in vitro methods for assessing whether a new drug candidate inhibits the $I_{Kr}$. Most in vitro experiments can investigate exposure to high concentrations of a test compound, but cannot reflect the metabolites or hemodynamic effects of the compound. In particular, hERG assays may miss the effects of a compound on other ion targets. Therefore, it is very important to assess electrocardiographs in vivo when evaluating drug-induced QT prolongation, since the QT interval is affected by many factors, including HR, plasma electrolytes, the autonomic nervous tone, and so on, and many drugs also affect these factors.

To elucidate the usefulness of non-clinical animal studies and construct a database of electrocardiograms in animals, which can be referred to in the developmental stage of new chemical entities, we examined the effects of some compounds in conscious dogs and monkeys that were chronically instrumented for telemetry transmitters. Monkeys have often been used in toxicology studies and more recently used in safety pharmacology studies. Although monkeys have usually been used to assess the cardiovascular system in the safety pharmacology studies, drug-induced QT prolongation in conscious unrestrained cynomolgus monkeys has not yet been sufficiently demonstrated. This review introduces data for the cardiovascular system in conscious, unrestrained cynomolgus monkeys, obtained using 10 positive reference compounds and 6 negative reference compounds in experiments conducted at 7 different facilities. The safety of each compound was assessed by integrating the non-clinical, clinical, and other relevant information. (Please note that the results from the present studies did not certify the safety of the compounds in humans.)

Materials and Methods

The current studies were conducted using a standardized protocol, which met the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society and the Guidelines for Animal Experimentation of the institution at each testing facility.

Standard protocol

The telemetry system (Data Science International, Arden Hills, MN, USA) that was used consisted of a transmitter (TL11M2-D70-PCT), a receiver (RMC-1), and a data acquisition system (ART/Siver) as well as a data analysis system [HEM 3.4 (Notocord SA, Paris, France) or Ponemah (Gould Instrument Systems, Inc., Valley View, OH, USA)]. The telemetry transmitter was implanted in the abdominal cavity of the monkeys under sodium pentobarbital (30 mg/kg, i.v.) anesthesia. A catheter with a pressure sensor for measuring blood
pressure was inserted into the abdominal aorta. The ECG electrodes were implanted subcutaneously into the right thorax and left abdomen (lead II configuration) through a subcutaneous tunnel. The monkeys were treated with antibiotics (benzylpenicillin potassium; Meiji Seika Kaisha, Tokyo) after surgery, and allowed a sufficient recovery period.

Four male cynomolgus monkeys weighing 3.0 – 7.0 kg were housed in individual stainless steel cages. Approximately 100 g of solid diet was supplied daily, and drinking water was provided ad libitum. Animals were fasted overnight before administration of a test compound. On the day of the test compound administration, the diet was supplied at 3 h after the administration.

Each monkey received an oral administration of the vehicle and 3 doses of a test compound in a dose-escalation or Latin Square design at 1-week intervals. Each compound was prepared as a suspension in 0.5 w/v% Methyl Cellulose 400 solution (Wako Pure Chemical Industries, Ltd., Osaka), and 5 mL/kg of the suspension was administered orally at approximately 10:00. After completion of a series of experiments for evaluating the effects of a test compound, dl-sotalol (Sotacol; Bristol Myers Squibb Company, New York, NY, USA) was administered using the same test procedure to ratify the present study. During the experiments, the telemetry signals from each monkey were recorded over a 24-h period following drug administration. Mean blood pressure (MBP), HR, and ECG parameters were measured, and blood samples were taken to analyze the plasma level of the test compound if possible. The sampling time points were determined according to the kinetic properties of each test compound, which were shown in previous reports.

The telemetry signals were averaged over 30 s to calculate MBP and HR. PR, QT, RR intervals, and QRS duration were averaged over 10 heartbeats. The corrected QT (QTcB) interval was calculated using Bazett’s formula [QTcB = (QT/(RR/1000)½)].

The effects of a test compound were evaluated using the percentage changes from the vehicle average baseline values. The vehicle average baseline values were calculated individually by averaging all the values for a particular parameter obtained after the vehicle treatment. The percentage changes from the vehicle average baseline values were calculated individually for all assay points in all groups, including the vehicle and 3 doses of the test compound. The mean percentage changes from the vehicle average baseline values were compared statistically between the vehicle and each dose of the test compound. Differences in the percentage changes between the vehicle and each dose of the test compound were then calculated individually, and their group mean differences, which represent the rates of prolongation by the test compound, were also calculated.

**Rationale for the doses of each compound**

Due to a lack of sufficient background data in the monkeys, the doses at which QT prolongation was predicted to occur were set at or above the effective doses reported in clinical and/or non-clinical studies. The doses and origins of the test compounds and the breeders of the animals are shown in Appendix 3.

**Determination of the plasma concentrations of the test compounds**

Blood was collected from the cephalic vein using a heparinized syringe for each test compound at the time points prescribed in the study protocol. The plasma concentrations of astemizole, cisapride, E-4031, and thioridazine were measured by HPLC, with reference to some issues regarding measurements of cisapride (3 – 5) and thioridazine (6 – 8). The plasma concentration of desmethylastemizole, which is a metabolite of astemizole (9) that inhibits I_{Kr} channels, was also measured by HPLC.

The plasma concentrations of bepridil and terfenadine (10,11) were determined using LC/MS/MS [HP1100 (Agilent Technologies, Aalo Alto, CA, USA), API 365 and API 3000 (Applied Biosystems/MDS SCIEX, Foster City, CA, USA), or 7000 Series (Hitachi, Ltd., Tokyo)].

The plasma concentration of haloperidol was measured using a commercial kit (MARKIT-G Haloperidol, Alfresa Pharma Corp., Osaka), and that of quinidine was measured by a fluorescence polarization immunoassay (FPIA) with a TDX Analyzer (Abbott Japan, Tokyo).

**Statistical analyses**

The data were expressed as the mean ± standard deviation (S.D.). Repeated-measures ANOVA with the Latin square or dose-escalation design was conducted for the cardiovascular parameters. A Dunnett-type multiple comparison test was conducted for comparisons between each dose of a compound and the vehicle using the model variance. When a certain dose level was significant or the dose × time interaction was significant, the effects at a certain dose or all doses at each time point were tested by t-tests. Values of P<0.05 were regarded as statistically significant. SAS software (Version 8.02; SAS Institute, Cary, NC, USA) was used for the data analysis.
Results

Circadian rhythms of HR, QT interval, and QTcB interval

HR, QT interval, and the QTcB interval in non-treated cynomolgus monkeys (n = 24) were measured every 6 min over a 24-h period. The mean heart rates were 125.2 ± 35.3 (range: 103 – 178) beats/min during the whole 24-h period, 138.9 ± 13.6 beats/min during the light cycle (07:00 – 19:00), and 111.6 ± 3.6 beats/min during the dark cycle (19:00 – 07:00) (Fig. 1a). The mean QT intervals were 240.8 ± 42.3 (range: 196.8 – 271.4) ms during the whole 24-h period, 221.5 ± 12.7 ms during the light cycle, and 260.0 ± 6.1 ms during the dark cycle (Fig. 1b). The mean QTcB intervals were 337.2 ± 27.1 (range: 314.3 – 354.1) ms during the whole day, 329.5 ± 6.9 ms during the light cycle, and 344.9 ± 3.8 ms during the dark cycle (Fig. 1c).

Pre-dose values of MBP, PR interval, and QRS duration

The circadian rhythms of MBP, PR interval, and QRS duration were not analyzed in this study. However, at approximately 09:00, the pre-dose values of MBP, PR interval, and QRS duration obtained from the non-treated cynomolgus monkeys (n = 24) were 91.8 ± 14.0 mmHg, 82.2 ± 13.4 ms, and 32.8 ± 6.5 ms, respectively.

Effects of test compounds on HR, MBP, ECG, and changes in plasma concentration of the compounds

Positive compounds: Astemizole (3, 10, and 30 mg/kg, p.o.; Table 1, Figs. 2 and 4, Appendices 1-1, 2-1, and 3-1): Astemizole at 30 mg/kg statistically significantly decreased MBP at 8 and 24 h, prolonged the PR interval at 6 h, and prolonged QRS duration at 3 and 6 h. The QT interval was significantly prolonged at 3 and 6 h at 10 mg/kg and at 3 – 24 h at 30 mg/kg. The QTcB interval was significantly prolonged at 1 – 8 h at 10 mg/kg and at 1 and 6 – 24 h at 30 mg/kg. Astemizole induced ventricular tachycardia in 2 of 4 monkeys at 10 mg/kg. In the 30 mg/kg group, premature ventricular contractions were found in 3 of 4 monkeys and 1 monkey showed TdP at 24 h after administration. The maximum percentage changes in the QTcB interval from the time-matched vehicle value at 3, 10, and 30 mg/kg were 6%, 27%, and 39%, respectively.

The plasma concentration of astemizole was below the limit of quantification at 3 mg/kg. Moreover, the plasma concentrations were only 0.01 µg/mL at 1 h at 10 mg/kg and 0.02 µg/mL at 1 – 3 h at 30 mg/kg. On the other hand, the plasma concentrations of desmethylastemizole, the main metabolite of astemizole, increased in a dose-dependent manner. The C_{max} and T_{max} were 0.03 ± 0.01 µg/mL at 1 h at 3 mg/kg, 0.23 ± 0.04 µg/mL at 3 h at 10 mg/kg, and 0.47 ± 0.12 µg/mL at 3 h
Table 1. Statistical significance, maximum group-mean-difference in QTcB interval, and values of pharmacokinetic parameters after oral administration of positive compounds in conscious monkeys

<table>
<thead>
<tr>
<th>Dosage levels (mg/kg)</th>
<th>Statistical significance</th>
<th>Maximum group-mean-difference in QTcB interval prolongation (%) and the time points (h)</th>
<th>C_{max} (ng/mL)</th>
<th>T_{max} (h)</th>
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<tr>
<td><strong>Astemizole (Desmethylastemizole)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3</td>
<td>NS</td>
<td>6 ± 5</td>
<td>1</td>
<td>BLQ (30)</td>
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<tr>
<td>10</td>
<td>S</td>
<td>27 ± 25</td>
<td>3</td>
<td>10 (230)</td>
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<tr>
<td>30</td>
<td>S</td>
<td>39</td>
<td>6</td>
<td>20 (470)</td>
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<tr>
<td><strong>Bepridil</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>10</td>
<td>NS</td>
<td>4 ± 7</td>
<td>1</td>
<td>173</td>
</tr>
<tr>
<td>30</td>
<td>S</td>
<td>13 ± 7</td>
<td>2</td>
<td>219</td>
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<tr>
<td>100</td>
<td>S</td>
<td>10 ± 17</td>
<td>4</td>
<td>230</td>
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<td><strong>Cisapride</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>1</td>
<td>NS</td>
<td>4 ± 6</td>
<td>3</td>
<td>33</td>
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<tr>
<td>3</td>
<td>S</td>
<td>9 ± 12</td>
<td>1</td>
<td>121</td>
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<td>10</td>
<td>S</td>
<td>17 ± 15</td>
<td>3</td>
<td>420</td>
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<td><strong>E-4031</strong></td>
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<td>0.3</td>
<td>S</td>
<td>29 ± 17</td>
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<td>35</td>
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<tr>
<td>1</td>
<td>S</td>
<td>44 ± 8</td>
<td>0.5</td>
<td>106</td>
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<tr>
<td>3</td>
<td>S</td>
<td>46 ± 16</td>
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<td><strong>Haloperidol</strong></td>
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<tr>
<td>0.3</td>
<td>NS</td>
<td>–2 ± 2</td>
<td>2</td>
<td>2</td>
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<tr>
<td>1</td>
<td>NS</td>
<td>3 ± 2</td>
<td>8</td>
<td>4</td>
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<tr>
<td>3</td>
<td>NS</td>
<td>8 ± 16</td>
<td>4</td>
<td>13</td>
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<td><strong>MK-499</strong></td>
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<tr>
<td>0.003</td>
<td>NS</td>
<td>6 ± 2</td>
<td>2</td>
<td>NT</td>
</tr>
<tr>
<td>0.03</td>
<td>NS</td>
<td>9 ± 4</td>
<td>2</td>
<td>NT</td>
</tr>
<tr>
<td>0.3</td>
<td>S</td>
<td>18 ± 13</td>
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<td>NS</td>
<td>15 ± 15</td>
<td>3</td>
<td>NT</td>
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<tr>
<td>10</td>
<td>NS</td>
<td>32 ± 14</td>
<td>6</td>
<td>NT</td>
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<td><strong>Quinidine</strong></td>
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<tr>
<td>2</td>
<td>NS</td>
<td>2 ± 2</td>
<td>8</td>
<td>275</td>
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<tr>
<td>10</td>
<td>S</td>
<td>9 ± 3</td>
<td>1</td>
<td>2100</td>
</tr>
<tr>
<td>50</td>
<td>S</td>
<td>15 ± 4</td>
<td>3</td>
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<td><strong>Terfenadine</strong></td>
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<tr>
<td>10</td>
<td>S</td>
<td>8 ± 4</td>
<td>24</td>
<td>9.6</td>
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<tr>
<td>30</td>
<td>NS</td>
<td>5 ± 6</td>
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<td>15.7</td>
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<td>24</td>
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<tr>
<td>5</td>
<td>NS</td>
<td>9 ± 10</td>
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<tr>
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<td>NS</td>
<td>14 ± 4</td>
<td>8</td>
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<tr>
<td>20</td>
<td>NS</td>
<td>11 ± 10</td>
<td>24</td>
<td>332</td>
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</table>

Differences in percentage change in QTcB interval between the vehicle and each dose of the test compounds were calculated individually, and the difference values were averaged at each time point to obtain the group-mean-difference values. The maximum group-mean-difference was generally shown as means ± S.D. S: Significant difference from the vehicle value. NS: No significant difference from the vehicle value. NT: Not tested. BLQ: Below limit of quantitation
The oral administration of cisapride (10 mg/kg) was observed at 1–4 h at 30 mg/kg and at 2–4 h at 100 mg/kg. The QTcB interval was also prolonged at 1 h at 3 mg/kg and at 1 and 3 h at 10 mg/kg. The QT and the QTcB intervals were prolonged in response to the plasma levels of cisapride. The maximum percentage change in the QTcB interval from the time-matched vehicle value at 1, 3, and 10 mg/kg were 4%, 9%, and 17%, respectively. The T\(_{\text{max}}\) at each dose was 1 h and the C\(_{\text{max}}\) values were 33 ± 10 ng/mL at 1 mg/kg, 121 ± 23 ng/mL at 3 mg/kg, and 420 ± 70 ng/mL at 10 mg/kg.

E-4031 (0.3, 1, and 3 mg/kg, p.o.; Table 1, Fig. 4, Appendices 1-4, 2-4, and 3-4): E-4031 had no effects on MBP, HR, PR interval, or QRS duration, but significantly prolonged the QT intervals at 0.5–2 h at 0.3 mg/kg and at 0.5–8 h at 1 and 3 mg/kg. The QTcB interval was prolonged at 0.5–3 h at 0.3 mg/kg and 0.5–8 h at 1 and 3 mg/kg. The maximum percentage changes in the QTcB interval from the time-matched vehicle value at 0.3, 1, and 3 mg/kg were 29%, 44%, and 46%, respectively. Arrhythmia was not found in any of the monkeys. The plasma concentrations of E-4031 increased in a dose-dependent manner and reached the T\(_{\text{max}}\) at 1 h for each dose. The C\(_{\text{max}}\) was 35 ± 8 ng/mL at 0.3 mg/kg, 106 ± 25 ng/mL at 1 mg/kg, and 433 ± 128 ng/mL at 3 mg/kg.

Haloperidol (0.3, 1, and 3 mg/kg, p.o.; Table 1, Fig. 4, Appendices 1-5, 2-5, and 3-5): Haloperidol did not affect MBP, HR, PR interval, or QRS duration. The QT interval was significantly shortened at 4–24 h at 0.3 mg/kg, but QTcB interval was not affected at any dose. The maximum percentage change in the QTcB interval from the time-matched vehicle value at 0.3, 1, and 3 mg/kg were −2%, 3%, and 8%, respectively. The C\(_{\text{max}}\) and T\(_{\text{max}}\) were 2 ± 2 ng/mL at 4 h at 0.3 mg/kg, 4 ± 1 ng/mL at 2 and 4 h at 1 mg/kg, and 13 ± 2 ng/mL at 2 h at 3 mg/kg, respectively.

MK-499 (0.003, 0.03, and 0.3 mg/kg, p.o.; Table 1, Fig. 4, Appendices 1-6, 2-6, and 3-6): No significant change was detected in MBP, HR, PR interval, QRS duration, or QT interval at any test dose, whereas the QTcB interval was statistically significantly prolonged at 1–8 h at 0.3 mg/kg. The maximum percentage change in the QTcB interval from the time-matched vehicle value at 0.3, 1, and 3 mg/kg were 2%, 3%, and 8%, respectively. The C\(_{\text{max}}\) and T\(_{\text{max}}\) were 2 ± 2 ng/mL at 4 h at 0.3 mg/kg, 4 ± 1 ng/mL at 2 and 4 h at 1 mg/kg, and 13 ± 2 ng/mL at 2 h at 3 mg/kg, respectively.

Cisapride (1, 3, and 10 mg/kg, p.o.; Table 1, Figs. 3 and 4, Appendices 1-3, 2-3, and 3-3): Cisapride had no effects on MBP, HR, PR interval, and QRS duration at any dose, but two patterns of arrhythmia were observed at 10 mg/kg (Fig. 3). The two types of arrhythmia were a premature ventricular contraction with a right bundle branch block in one monkey and a paroxysmal atrial tachycardia in the other monkey. As HR increased, the PR interval and QRS duration seemed to prolong during the occurrence of the arrhythmia at 1 h; the parameters of ECG could not be measured correctly at that time. Statistically significant prolongation was observed in the QT interval at 3–8 h at 10 mg/kg. The QTcB interval was also prolonged at 1 h at 3 mg/kg and at 1 and 3 h at 10 mg/kg. The QT and the QTcB intervals were prolonged in response to the plasma levels of cisapride. The maximum percentage change in the QTcB interval from the time-matched vehicle value at 1, 3, and 10 mg/kg were 4%, 13%, and 10%, respectively. The T\(_{\text{max}}\) at each dose was 1 h and the C\(_{\text{max}}\) values were 173 ± 87 ng/mL at 1 h at 10 mg/kg, 219 ± 188 ng/mL at 1 h at 30 mg/kg, and 230 ± 132 ng/mL at 4 h at 100 mg/kg. Emesis was observed in all animals at a dose of 100 mg/kg.

Bepridil (10, 30, and 100 mg/kg, p.o.; Table 1, Fig. 4, Appendices 1-2, 2-2, and 3-2): Bepridil did not affect MBP, HR, PR interval, or QRS duration at any dose. A statistically significant prolongation in the QT interval was observed at 1–4 h at 30 mg/kg and at 4 h at 100 mg/kg. The QTcB interval was also prolonged at 2–4 h and 24 h at 30 mg/kg and at 2–4 h at 100 mg/kg. The maximum percentage changes in the QTcB interval from the time-matched vehicle value at 10, 30, and 100 mg/kg were 4%, 13%, and 10%, respectively. The C\(_{\text{max}}\) and T\(_{\text{max}}\) were 173 ± 87 ng/mL at 1 h at 10 mg/kg, 219 ± 188 ng/mL at 1 h at 30 mg/kg, and 230 ± 132 ng/mL at 4 h at 100 mg/kg. Emesis was observed in all animals at a dose of 100 mg/kg.

Cisapride (1, 3, and 10 mg/kg, p.o.; Table 1, Figs. 3 and 4, Appendices 1-3, 2-3, and 3-3): Cisapride had no effects on MBP, HR, PR interval, and QRS duration at any dose, but two patterns of arrhythmia were observed at 10 mg/kg (Fig. 3). The two types of arrhythmia were a premature ventricular contraction with a right bundle branch block in one monkey and a paroxysmal atrial tachycardia in the other monkey. As HR increased, the PR interval and QRS duration seemed to prolong during the occurrence of the arrhythmia at 1 h; the parameters of ECG could not be measured correctly at that time. Statistically significant prolongation was observed in the QT interval at 3–8 h at 10 mg/kg. The QTcB interval was also prolonged at 1 h at 3 mg/kg and at 1 and 3 h at 10 mg/kg. The QT and the QTcB intervals were prolonged in response to the plasma levels of cisapride. The maximum percentage change in the QTcB interval from the time-matched vehicle value at 1, 3, and 10 mg/kg were 4%, 9%, and 17%, respectively. The T\(_{\text{max}}\) at each dose was 1 h and the C\(_{\text{max}}\) values were 33 ± 10 ng/mL at 1 mg/kg, 121 ± 23 ng/mL at 3 mg/kg, and 420 ± 70 ng/mL at 10 mg/kg. E-4031 (0.3, 1, and 3 mg/kg, p.o.; Table 1, Fig. 4, Appendices 1-4, 2-4, and 3-4): E-4031 had no effects on MBP, HR, PR interval, or QRS duration, but significantly prolonged the QT intervals at 0.5–2 h at 0.3 mg/kg and at 0.5–8 h at 1 and 3 mg/kg. The QTcB interval was prolonged at 0.5–3 h at 0.3 mg/kg and 0.5–8 h at 1 and 3 mg/kg. The maximum percentage changes in the QTcB interval from the time-matched vehicle value at 0.3, 1, and 3 mg/kg were 29%, 44%, and 46%, respectively. Arrhythmia was not found in any of the monkeys. The plasma concentrations of E-4031 increased in a dose-dependent manner and reached the T\(_{\text{max}}\) at 1 h for each dose. The C\(_{\text{max}}\) was 35 ± 8 ng/mL at 0.3 mg/kg, 106 ± 25 ng/mL at 1 mg/kg, and 433 ± 128 ng/mL at 3 mg/kg. Haloperidol (0.3, 1, and 3 mg/kg, p.o.; Table 1, Fig. 4, Appendices 1-5, 2-5, and 3-5): Haloperidol did not affect MBP, HR, PR interval, or QRS duration. The QT interval was significantly shortened at 4–24 h at 0.3 mg/kg, but QTcB interval was not affected at any dose. The maximum percentage change in the QTcB interval from the time-matched vehicle value at 0.3, 1, and 3 mg/kg were −2%, 3%, and 8%, respectively. The C\(_{\text{max}}\) and T\(_{\text{max}}\) were 2 ± 2 ng/mL at 4 h at 0.3 mg/kg, 4 ± 1 ng/mL at 2 and 4 h at 1 mg/kg, and 13 ± 2 ng/mL at 2 h at 3 mg/kg, respectively. MK-499 (0.003, 0.03, and 0.3 mg/kg, p.o.; Table 1, Fig. 4, Appendices 1-6, 2-6, and 3-6): No significant change was detected in MBP, HR, PR interval, QRS duration, or QT interval at any test dose, whereas the QTcB interval was statistically significantly prolonged at 1–8 h at 0.3 mg/kg. The maximum percentage change in the QTcB interval from the time-matched vehicle value at 0.3, 1, and 3 mg/kg were 2%, 3%, and 8%, respectively. The C\(_{\text{max}}\) and T\(_{\text{max}}\) were 2 ± 2 ng/mL at 4 h at 0.3 mg/kg, 4 ± 1 ng/mL at 2 and 4 h at 1 mg/kg, and 13 ± 2 ng/mL at 2 h at 3 mg/kg, respectively.
changes in the QTcB interval from the time-matched vehicle value at 0.003, 0.03, and 0.3 mg/kg were 6%, 9%, and 18%, respectively.

*Pimozide* (0.1, 1, and 10 mg/kg, p.o.; Table 1, Fig. 4, Appendices 1-7, 2-7, and 3-7): Pimozide had no statistically significant effects on any of the measured parameters. Although the standard deviations for HR were very large, tachycardia seemed to occur at 0.1 and 10 mg/kg. Moreover, the QTcB interval tended to be prolonged at 3 – 24 h at 10 mg/kg, with a maximal change of 32 ± 14% at 6 h. The maximum percentage changes in the QTcB interval from the time-matched vehicle value at 0.1, 1, and 10 mg/kg were −1%, 15%, and 32%, respectively.

*Quinidine* (2, 10, and 50 mg/kg, p.o.; Table 1, Fig. 4, Appendices 1-8, 2-8, and 3-8): There were no significant changes in MBP, HR, PR interval, or QRS duration after oral administration of quinidine at any test dose. Quinidine prolonged the QT interval at 0.5 and 2 – 4 h at 50 mg/kg. The QTcB interval was also prolonged at 0.5 – 2 h at 10 mg/kg and at 0.5 – 4 h at 50 mg/kg. The maximum percentage changes in the QTcB interval from the time-matched vehicle value at 2, 10, and 50 mg/kg were 2%, 9%, and 15%, respectively. The T_{max} was 2 h at each dose; and the C_{max} values were 275 ± 189 ng/mL at 2 mg/kg, 2100 ± 845 ng/mL at 10 mg/kg, and 5850 ± 2689 ng/mL at 50 mg/kg.

*Terfenadine* (10, 30, and 100 mg/kg, p.o.; Table 1, Fig. 4, Appendices 1-9, 2-9, and 3-9): Terfenadine had no effects on HR, PR interval, QRS duration, or QT interval, but significantly decreased MBP at 2 – 4 h and 8 h at 100 mg/kg and prolonged the QTcB interval at

<table>
<thead>
<tr>
<th>Dosage levels (mg/kg)</th>
<th>Statistical significance</th>
<th>Maximum group-mean-difference in QTcB interval prolongation (%) and the time points (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>NS</td>
<td>4 ± 5, 8</td>
</tr>
<tr>
<td>10</td>
<td>NS</td>
<td>10 ± 8, 8</td>
</tr>
<tr>
<td>100</td>
<td>NS</td>
<td>−1 ± 13, 8</td>
</tr>
<tr>
<td>Captopril</td>
<td>NS</td>
<td>5 ± 6, 8</td>
</tr>
<tr>
<td>3</td>
<td>NS</td>
<td>4 ± 3, 8</td>
</tr>
<tr>
<td>100</td>
<td>NS</td>
<td>1 ± 7, 3</td>
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<td>Ciprofloxacin</td>
<td>NS</td>
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<tr>
<td>30</td>
<td>NS</td>
<td>5 ± 14, 24</td>
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<tr>
<td>100</td>
<td>NS</td>
<td>5 ± 12, 24</td>
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<tr>
<td>Diphenhydramine</td>
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<tr>
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<td>NS</td>
<td>4 ± 4, 6</td>
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<tr>
<td>10</td>
<td>NS</td>
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<tr>
<td>Propranolol</td>
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<td>3</td>
<td>NS</td>
<td>3 ± 2, 8</td>
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<tr>
<td>30</td>
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<td>NS</td>
<td>3 ± 2, 1</td>
</tr>
<tr>
<td>15</td>
<td>S</td>
<td>5 ± 4, 2</td>
</tr>
</tbody>
</table>

Differences in the percentage change in QTcB interval between the vehicle and each dose of the test compounds were calculated individually, and the difference values were averaged at each time point to obtain the group-mean-difference values. The maximum group-mean-difference was shown as means ± S.D. S: significant difference from the vehicle value. NS: no significant difference from the vehicle value.
Terfenadine was not found in the plasma of 3 of 4 monkeys. The $C_{\text{max}}$ and $T_{\text{max}}$ of the remaining monkey were 9.6 ng/mL at 2 h at 10 mg/kg, 15.7 ng/mL at 4 h at 30 mg/kg, and 19.8 ng/mL at 4 h at 100 mg/kg. No prolongations of the QT and the QTcB intervals were observed in this monkey.

**Thioridazine (5, 10, and 20 mg/kg, p.o.; Table 1, Fig. 4, Appendices 1-10, 2-10, and 3-10):** Thioridazine had no effects on MBP, PR interval, or QRS duration. Thioridazine significantly increased HR in a dose-dependent manner at 1 and 3 h at 5 mg/kg, at 1 – 6 h at 10 mg/kg, and at 1 – 8 h at 20 mg/kg. The QT interval tended to shorten according to the increases in HR at the same time points, but no significant differences in the QTcB interval were seen at any of the doses or time points. The maximum percentage changes in the QTcB interval seen at any of the doses or time points. The maximum percentage changes in the QTcB interval from the time-matched vehicle value at 5, 10, and 20 mg/kg were 9%, 14%, and 11%, respectively. The $T_{\text{max}}$ and $C_{\text{max}}$ were 1 h and 265 ± 176 ng/mL at 5 mg/kg, 6 h and 316 ± 203 ng/mL at 10 mg/kg, and 6 h and 332 ± 205 ng/mL at 20 mg/kg, respectively. Dose-dependency of the maximum plasma concentrations after administration of thioridazine at 5 – 20 mg/kg was not observed.

**Negative compounds:** *Aspirin* (10, 30, and 100 mg/kg, p.o.; Table 2, Fig. 4, Appendices 1-11, 2-11, and 3-11): Aspirin had no effects on MBP, HR, PR, QRS, QT, and QTcB intervals at any dose. The maximum percentage changes in the QTcB interval from the time-matched vehicle value at 10, 30, and 100 mg/kg were 4%, 10%, and 1%, respectively.

**Captopril (3, 10, and 100 mg/kg, p.o.; Table 2, Fig. 4, Appendices 1-12, 2-12, and 3-12):** No significant change was detected in MBP, HR, PR interval, QRS duration, QT interval, and QTcB interval after oral administration of captopril at any test dose. The maximum percentage changes in the QTcB interval from the time-matched vehicle value at 3, 10, and 100 mg/kg were 5%, 4%, and 1%, respectively.

**Ciprofloxacin (30, 100, and 300 mg/kg, p.o.; Table 2, Fig. 4, Appendices 1-13, 2-13, and 3-6):** There were no significant change in MBP, HR, PR interval, QRS duration, QT interval, and QTcB interval after oral administration of ciprofloxacin at any test dose. The maximum percentage changes in the QTcB interval from the time-matched vehicle value at 30, 100, and 300 mg/kg were 2%, 5%, and 5%, respectively.

**Diphenhydramine (1, 3, and 10 mg/kg, p.o.; Table 2, Fig. 4, Appendices 1-14, 2-14, and 3-13):** No significant change was detected in PR interval, QRS duration, QT

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**Fig. 4.** Relationships between the percent value of QTcB interval prolongation and doses in 16 test compounds. Negative compounds and positive compounds are described by dashed and solid lines, respectively.
interval, and QTcB interval after oral administration of diphenhydramine at any test dose. Statistically significant differences were observed occasionally in MBP and HR. The maximum percentage changes in the QTcB interval from the time-matched vehicle value at 1, 3, and 10 mg/kg were 2%, 4%, and 7%, respectively.

Propranolol (3, 10, and 30 mg/kg, p.o.; Table 2, Fig. 4, Appendices 1-15, 2-15, and 3-9): Propranolol statistically significantly decreased HR at 1 h at 3 mg/kg, at 1 – 8 h at 10 mg/kg, and at 1 – 24 h at 30 mg/kg, respectively. The PR interval was prolonged at 1 and 4 h at 3 mg/kg, at 1 – 4 h at 10 and 30 mg/kg, respectively. The QT interval was prolonged at 2 – 8 h at 10 mg/kg and at 2 – 24 h at 30 mg/kg, but the QTcB interval prolongation was observed only at 24 h at 30 mg/kg. The maximum percentage changes in the QTcB interval from the time-matched vehicle value at 3, 10, and 30 mg/kg were 2%, 3%, and 5%, respectively.

Verapamil (1, 5, and 15 mg/kg, p.o.; Table 2, Fig. 4, Appendices 1-16, 2-16, and 3-4): Although verapamil increased HR at 0.5 – 2 h at 15 mg/kg, there were no effects on MBP, PR interval, QRS duration, or QT interval at any of the test doses. Statistically significant differences were observed in the QTcB interval at 2 h at 15 mg/kg. The maximum percentage changes in the QTcB interval from the time-matched vehicle value at 1, 5, and 15 mg/kg were 4%, 3%, and 5%, respectively.

The relationship between doses of test compounds and the QTcB interval prolongation are summarized in Tables 1 and 2 and Fig. 4. The maximum prolongation rate in the negative compounds was 10% and the minimum prolongation with a statistically significant difference in the positive compounds was also 10%.

Discussion

Circadian rhythms for MBP and electrophysiological parameters in cynomolgus monkeys

This study may be the first to report cardiac hemodynamic and electrophysiological data using a telemetry system in cynomolgus monkeys with the same experimental protocol obtained at different testing facilities.

Since the blood collection procedure is considered to affect the cardiovascular and ECG parameters, preliminary studies were conducted in some of the facilities before setting up a standard protocol. From the results of these preliminary studies, we decided to collect blood samples at intervals of longer than 1 h in general and to correct the QT interval data using Bazett’s formula. Furthermore, the responses of the QTcB intervals to oral administration of dl-sotalol with repeated blood collections were confirmed to be stable and TdP was found in 1 monkey at 10 mg/kg of dl-sotalol. Therefore, 5 mg/kg of dl-sotalol was used at the ratification for each study. Maximal percentage ranges of the QTcB interval in all facilities were from 10% to 23%. These results suggest that the QTcB data from every facility was acceptable.

Regarding circadian rhythms, the mean HR was 139 beats/min during the light cycle (07:00 to 19:00) and 111 beats/min during the dark cycle (19:00 to 07:00). Although high heart rates and short QT intervals were observed at some time points, the QTcB interval was corrected properly by Bazett’s formula. However, only the QTcB interval in the light cycle was analyzed to avoid false positive prolongation in this study, since the QTcB interval in the dark cycle was longer (344.9 ms) than that in the light cycle (329.5 ms).

Moreover, in the light cycle, the scattering of the QTcB intervals from the control baseline values in 4 animals in different testing facilities was within 10% (12). This observation supports the results that a more than 10% change in the QTcB interval compared with the control baseline is considered to be caused by a test compound.

The MBP, PR interval, and QRS duration are considered to be stable parameters since almost the same values were found in the same animals, even on different testing days (data not shown).

These findings suggest that electrocardiograms including the QT interval and blood pressure can be measured consistently using the telemetry method in cynomolgus monkeys. Thus, this monkey model is very useful for investigating drug-induced QT prolongation under the standard protocols of safety pharmacology studies.

Predictability of QTcB prolongation in cynomolgus monkey

Positive compounds: From Tables 1 and 2 and Fig. 4, the borderline in the QTcB interval prolongation rate between negative compounds and positive compounds is considered to be 10%.

Among the 10 positive compounds, QTcB prolongation was detected for 7 of them, but not for haloperidol, terfenadine, and thioridazine.

Astemizole, bepridil, cisapride, E-4031, MK-499, and quinidine significantly prolonged the QTcB intervals. These results were consistent with previous reports on in vitro studies (9, 13 – 20) and in vivo studies (21 – 28). In the case of pimozide, although in the change in the QTcB interval did not reach statistical significance, the percent change was large. Indeed, individual absolute values of the QTcB interval from pre-dose to 6 h after administration of 10 mg/kg of pimozide were from 340 ms to 519 ms, from 330 ms to 407 ms, and from
324 ms to 442 ms, respectively. The results confirm that pimozide prolongs the QTcB interval, and this is supported by previous reports (24, 29).

Haloperidol shortened the QT interval in the present study, but it did not affect the QTcB interval. The changes appeared to occur coincidentally and the values of the changes were within the physiological range (Fig. 1). A plasma haloperidol concentration of 13 ng/mL (34.6 nmol/L) in the monkeys was considered to be in the effective concentration range of 8 – 18 ng/mL in clinical use (30), and the plasma protein binding rate of haloperidol was reported to be 92% in humans (31). If the binding rate in monkeys is taken to be the same as that in humans, the free plasma concentration is about 2.8 nmol/L. Since haloperidol was found to inhibit hERG currents with an IC_{50} value of 27 nmol/L (31), the plasma values did not reach the hERG IC_{50}. These results suggest that haloperidol does not affect the QT interval at single oral doses of up to 3 mg/kg (13 ng/mL) and that the plasma free concentration, which was about 1/10 the level of the hERG IC_{50}, could not prolong the QTc interval in cynomolgus monkeys.

Terfenadine had no effects on HR, PR interval, QRS duration, and QT interval at any of the tested doses, but statistically significant differences were found in MBP and QTcB interval in some points. These changes were not related to the treatment with terfenadine, because a detectable plasma concentration of terfenadine was only found in 1 of 4 monkeys. Terfenadine is metabolized by CYP3A4 and ketoconazole is known to inhibit the metabolic enzyme (32). Terfenadine prolonged the QTcB interval in cynomolgus monkeys at 100 mg/kg, when it was given together with ketoconazole (50 mg/kg) at the same testing facility (data not shown). Ando et al. also reported that co-administration of 30 mg/kg of terfenadine and 100 mg/kg of ketoconazole prolonged the QTcB interval in cynomolgus monkeys, whereas the same dose of terfenadine alone had no effect on the QTcB interval (33). The reason why QT prolongation was hardly detected in cynomolgus monkeys remains unclear, but these observations suggest that terfenadine is rapidly processed to its metabolites, which are unable to prolong the QT interval, and that it may prolong the QT interval if its metabolism can be inhibited, thereby increasing its plasma concentration.

Thioridazine was reported to prolong the QTcB interval after a single oral dose of 50 mg/kg (34), prolong the APD in guinea pigs (15), and to block hERG currents (35). Thioridazine prolonged the QTcB interval to 14% at the middle dose. However, it was difficult to judge whether the value was thioridazine-induced prolongation or not, because the prolongation was not dose-dependent and tachycardia was observed at the middle dose. In conscious telemetered dogs, thioridazine at an oral dose of 5 mg/kg or above significantly prolonged the QT interval at a plasma concentration of 162 ng/mL (24). However, although thioridazine at 20 mg/kg exhibited a Cmax of 332 ng/mL, no prolongation of the QTcB interval was observed in the present monkey study. A possible reason for the failure to detect the QTcB prolongation by thioridazine in the present study is that the I_{Ks} channels were dominated by the thioridazine-induced tachycardia that masked the inhibitory effects on I_{Ks}. Moreover, it is speculated that the QTcB interval was not properly corrected by Bazett’s formula in the presence of tachycardia in this case.

**Negative compounds**: Aspirin and captopril had no effects on MBP, HR, PR interval, QRS duration, QT interval, and QTcB interval at any of the doses examined. Lack of the QTcB prolongation in the two compounds was well reflected in the results of hERG assays (36).

Ciprofloxacin and diphenhydramine did not prolong QTcB in the present study, although these drugs are known to inhibit the hERG current (31, 37) and prolong QTc interval in perfused guinea-pig hearts (38). The reason for the discrepancy between the present study and the hERG study in ciprofloxacin may be that the predictive plasma concentration (0.88 µg/mL) of the drug at 30 mg/kg (39) did not reach the hERG IC_{50} value (approximately 400 µg/mL) (36). Although the doses of diphenhydramine used in the present study were 1- to 10-fold higher than the human clinical doses, no apparent dose-dependent effect on the QTcB interval could be identified. Diphenhydramine has not been reported to cause TdP in clinical use. These facts suggest that hERG assays may overestimate the risks of QT prolongation, and the usefulness of in vivo monkey studies has been demonstrated for predicting QT prolongation.

Propranolol prolonged the QT interval due to bradycardia. The QTcB interval prolongation was very weak and seemed to occur incidentally. Judging from the results, propranolol had no effect on the QTcB interval in the monkey, although propranolol was reported to prolong the QT duration at an intravenous dose of 10 mg/kg in humans (40).

Verapamil increased HR, but did not markedly affect the QT or the QTcB interval in the monkeys. A statistically significant difference in the QTcB at 2 h seems to have been detected coincidentally. Although verapamil was reported to be a potent hERG blocker (IC_{50} = 143 nmol/L) (41), QTcB interval prolongation was not observed after verapamil administration in the present

monkey study. This result is supported by previous reports on guinea pigs (42) and humans (43).

These findings suggest that monkey telemetry assays can distinguish between negative compounds and positive compounds. However, it was difficult to predict QTcB interval prolongation in some positive compounds that affect HR and/or are peculiar in their metabolism in cynomolgus monkeys. Since a formula to calculate the correct QT interval has not been established for cynomolgus monkeys, further investigation is needed to develop such a formula. Regarding metabolisms in cynomolgus monkeys, data of plasma concentration and/or co-administration of metabolic inducers or inhibitors are highly supportive in judging whether a test compound prolongs the QTcB interval.

Comparison of the sensitivity in QT prolongation between cynomolgus monkey and other animals

The degree of QTc prolongation in the negative compounds was comparable between the cynomolgus monkeys and the beagle dogs (24). On the other hand, astemizole has also been reported to cause TdP in common marmosets at an oral dose of 30 mg/kg (22), but prolonged the QTcF interval (Fridericia correction) without any signs of arrhythmia in conscious telemetered dogs (24). Cisapride significantly prolonged the QTcB interval by 9% and 17% at plasma concentrations of 121 and 420 ng/mL, respectively. On the other hand, cisapride prolonged the QTcB interval in conscious telemetered dogs by 10% and 18% at plasma concentrations of 615 and 1551 ng/mL, respectively (24). Raunig et al. reported that it was difficult to detect cisapride-induced QT prolongation in conscious dogs (44). These results suggest that cisapride may prolong the QT interval at lower plasma concentrations in cynomolgus monkeys than in dogs. In conscious telemetered monkeys, E-4031 prolonged the QTc interval much more than that in dogs, when the plasma concentration of E-4031 was almost equal to those found in the monkeys and dogs (24). These results suggest that cynomolgus monkeys may be more sensitive for prolongation of the QTcB interval than beagle dogs.

Generation of arrhythmia

In the current study on cynomolgus monkeys, astemizole at an oral dose of 10 mg/kg or above prolonged the QT and the QTc intervals; and it also caused arrhythmia, premature ventricular contractions, and ventricular premature beats. Moreover, astemizole at 30 mg/kg caused TdP and astemizole-induced TdP has been reported in the common marmoset (22) and in humans (23). In 2 of 4 monkeys in the present study, arrhythmia occurred before the QTcB interval prolongation. The reasons for the generation of the arrhythmia are not clear, but occurrences of arrhythmia without the QTcB interval prolongation suggested that the cynomolgus monkey may be a species in which the cardiac repolarization reserve is not so large.

The arrhythmia observed for cisapride at 10 mg/kg was a premature ventricular contraction with a right bundle block in one monkey and a paroxysmal atrial tachycardia in another monkey, but neither were TdP. It was unclear whether the arrhythmia was a sign of TdP development. Although the mechanisms of the arrhythmia observed in the present study remain unclear, ventricular tachycardia has been reported to be caused by Ca2+-channel blockade in humans (45). Ca2+-channel blockers generally prolong the PR interval, and cisapride-induced PR interval prolongation has been reported in anesthetized dogs (46). Furthermore, cisapride was reported to inhibit the L-type calcium current in guinea pigs (47). The cisapride-induced tachycardia may have arisen by inhibition of the Ca2+-channel. Cisapride blocks hERG current with an IC50 value of 6.5 nmol/L (approximately 30.3 ng/mL) (16) and binds plasma protein at a rate of 95% in humans (48). The estimated free plasma concentration of cisapride in monkeys is likely much lower than the IC50 value. This finding suggests that monkey telemetry assays may be a sensitive model for predicting QT prolongation and/or a drug-induced arrhythmia.

Although E-4031 and pimozide induced marked QTcB interval prolongation, TdP or ventricular tachycardia did not appear like astemizole and cisapride in the present study. In anesthetized dogs, the effects of dofetilide and E-4031 on the IC50 currents differed from those of disopyramide on the electrical cardiac responses to vagus stimulation (49). Therefore, autonomic nerve tone may possibly affect QT prolongation and/or arrhythmogenesis. Further investigation into the relationship between the QT prolongation, autonomic nervous tone, and arrhythmia is needed in cynomolgus monkeys.

The plasma concentrations of the test compounds and their metabolites

Among the positive compounds used in these experiments, astemizole, cisapride, pimozide, and terfenadine were reported to be mainly metabolized by cytochrome P450 subtype CYP3A4, while haloperidol and thioridazine are metabolized by subtype CYP 2D6 in humans (50). However, there have been no reports regarding the metabolic enzymes for these compounds in monkeys.

In the cynomolgus monkey, the plasma concentration of desmethyastemizole was much higher than that of its
parent compounds, except for haloperidol, terfenadine, and thioridazine, caused prolonged QTcB interval, whereas none of the negative compounds had any effect on the QTcB interval. QTcB interval prolongation and arrhythmia were detected for astemizole and cisapride at relatively low plasma concentrations of the parent compound astemizole. On the other hand, plasma concentrations of astemizole and desmethylandastemizole were almost equal in dogs (24). These facts were probably the reason that arrhythmia occurred in monkeys but not in dogs.

Although terfenadine was hardly detected in the plasma in the present study, its plasma concentration and the QT interval both increased when terfenadine was co-administered with ketoconazole, a CYP 3A4 inhibitor, in cynomolgus monkeys (32). This report suggests that terfenadine is certainly metabolized by CYP 3A4 and that its metabolic rate in cynomolgus monkeys is very fast. Although the plasma concentration of haloperidol in the cynomolgus monkeys was also much lower than that in the beagle dogs (24), there is no report about CYP 2D6 in cynomolgus monkeys.

There were no regional differences in the total P-450 content or the major cytochrome P450 isozymes (CYP 1A, 2A, 2B, 2C, 2D, 2E1, and 3A4) in cynomolgus monkeys, although the variations among individuals were large (51). However, there are no reasons why toxicokinetics and/or pharmacokinetics in cynomolgus monkeys are different from beagle dogs and/or humans. These observations represent a limitation of studies using cynomolgus monkeys and strongly indicate the need for measurements of the plasma concentrations of test compounds in experiments, when cynomolgus monkeys are used for safety pharmacology studies and/or toxicology studies.

Almost all the positive compounds, except for haloperidol, terfenadine, and thioridazine, caused QTcB interval prolongation, whereas none of the negative compounds had any effect on the QTcB interval. QTcB interval prolongation and arrhythmia were detected for astemizole and cisapride at relatively low plasma concentrations compared to those in humans and dogs. E-4031 and pimozide markedly prolonged QTcB. Although attention should be paid to compounds for which the metabolites and/or metabolic rates differ between humans and monkeys and the HR changes are beyond the correctable range for the QTcB interval, when results according to the standard protocol satisfy the following condition that 1) QTcB interval is prolonged in a dose-dependent manner, 2) statistically significant differences are recognized, and 3) prolongation rate of QTcB interval is >10%, test compounds probably have sufficient potency to prolong the QTcB interval. In vivo QT assays using telemetry systems in conscious cynomolgus monkeys are considered to be a sensitive and useful model for assessing the potential for drug-induced QT prolongation in humans.

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